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Entitled

Temporal and Spatial Genetic Consistency

of Walleye (*Sander vitreus*) Spawning Groups

By

Jo Ann Banda

Submitted to the Graduate Faculty as partial fulfillment of the requirements for

the Master of Science Degree in Biology

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An Abstract of

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The population genetic structure of three of the largest walleye spawning groups in Lake Erie is tested for consistency over time and space spanning 14 years, based on nine high-resolution nuclear DNA microsatellite loci. Previous genetic studies focused on a one-time genetic "snapshot", with an earlier study by our laboratory finding that the genetic structure of three Lake Erie spawning groups along the southern shore - Maumee River, Sandusky River, and Van Buren Bay reefs - appeared similar in 2003, whereas most other spawning groups across Lake Erie were genetically distinctive. The present study analyzes the stability of genetic similarity patterns within and among 726 walleye spawning at these three sites across years and age cohorts in 1995, 1998, 2003, 2007, and 2008. Genetic patterns are evaluated using pairwise F_{ST} analog and contingency tests, AMOVA partitioning, and Bayesian assignment tests. Results reveal overall year-to-year consistency in genetic structure of walleye spawning at the three sites, with some annual variation in the Van Buren Bay reef group. Greater genetic divergence from the other groups is detected in the Van Buren Bay spawning group, which reflects greater geographic separation. Walleye spawning in the Sandusky and Maumee Rivers are

genetically distinguishable from each other when data from all years are combined, which suggests possible sample size effect (i.e., annual sample sizes likely were not large enough to detect their genetic differentiation). No significant differences occur among age cohorts, between the sexes, or among sampling dates within spawning runs. Results demonstrate the importance of sampling over several years of walleye spawning runs in order to resolve fine-scale genetic relationships within an open lake system.

This work is dedicated to the memories of Roswitha Albrecht and Margaret Esther Nye.

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Chapter 1

Introduction

The walleye *Sander vitreus* (Teleostei: Percidae) is one of the most ecologically and economically important exploited Great Lakes' fishes, with Lake Erie supporting its largest fishery (Fielder 2002; Hubbs and Lagler 2004) estimated as 2.1 million walleye harvested in 2011 (Lake Erie Committee - Walleye Task Group 2011). Maintaining healthy walleye stocks has been identified as key to Lake Erie habitat restoration efforts (Ryan et al. 2003) and management objectives (Reiss 2009). Since Lake Erie lacks obvious vicariant geographic divisions, there appears to be ample potential for this vagile species to interbreed. However, tagging studies have shown that although walleye commingle during non-spawning months, they tend to return to the same spawning locations with an apparently high degree of philopatry (Ferguson and Derksen 1971; Wolfert and Van Meter 1978).

Reproductive isolation of spawning groups can lead to formation of genetically distinct fishery stocks, which then adapt to local conditions over time (Horrall 1981; Hauser and Carvalho 2008). Hallerman et al. (2003) define a stock as, "a population

subunit that interbreeds freely in a given geographic location, whose individuals share a common gene pool, and differs significantly from other subunits." It is important to identify discrete Lake Erie walleye stocks, as management strategies and exploitation can variably impact them (Locke et al. 2005) and they may each contribute differentially to overall harvests. Genetic divergence is tested here to delineate population structure.

Genetic studies of walleye have shown both large-scale continental genetic divergence patterns that corresponded to their origins from glacial refugia and historic separations in major watersheds (Billington et al. 1992; Stepien et al. 2009), as well as discrete intralacustrine groups and fine-scale patterns among spawning groups from various Lake Erie tributary and reef sites (Ney 1978; Stepien 1995; Merker and Woodruff 1996; Stepien and Faber 1998; Strange and Stepien 2007; Stepien et al. 2009, 2010). Strange and Stepien (2007) discerned genetically divergent spawning groups of walleye across Lake Erie, which appeared especially distinctive in eastern basin rivers. They also identified greater genetic connectivity among some walleye spawning groups from 2003 along the southern lakeshore, extending from the western basin rivers (Maumee and Sandusky Rivers) to the near eastern basin reefs (Van Buren Bay). Since their data were based on a single year for this pattern, that genetic "snapshot" may or may not reflect a long-term pattern.

The present study seeks to provide insight into contemporary genetic population structure among walleye spawning groups by adding a temporal component and further testing the genetic connectivity pattern detected by Strange and Stepien (2007) for five years of spawning run data (1995, 1998, 2003, 2007, and 2008). Nine nuclear microsatellite loci (the same ones used by Strange and Stepien 2007) are used to test the

genetic variation within and among three of the largest Lake Erie spawning groups (Maumee River, Sandusky River, and Van Buren Bay reefs) along the southern shore over a span of 14 years. The following hypotheses (null/alternative) are tested, (1) population genetic compositions of walleye spawning groups are/are not consistent from year to year, (2) the degree of genetic connectivity and divergence relationships among walleye spawning groups are/are not consistent from year to year, (3) the genetic composition of males versus females does not differ/differs within a given spawning run, (4) the genetic composition of age cohorts does not differ/differs within a given spawning run, and (5) the genetic composition between sampling dates in a given year does not differ/differs within spawning runs.

1.1 History of Lake Erie walleye fishery

Commercial exploitation of walleye in the western basin increased after the collapse of the cisco's (*Coregonus atedi*) fishery in the 1940s and increased further following the collapse of the blue pike's (*Sander vitreus glaucus*) – a walleye variant - fishery in the central basin in the 1950s (Nepszy 1991). Intensified fishing effort, more efficient fishing gear, and degraded spawning habitats resulted in the decline of Lake Erie walleye following peak harvests in the mid 1990s (Nepszy 1977; Hatch et al. 1987; Roseman et al. 2002). Fewer walleye reside in the eastern Lake Erie basin (Hartman 1973), whose numbers have been comparably more consistent (Ryan et al. 2003). Commercial fisheries in western Lake Erie closed in 1970 and recreational anglers were prohibited from keeping Lake Erie walleye due to high levels of mercury in fish tissue (Hatch et al. 1987). In 1972, the sport fishery reopened in Ohio, Michigan, and Ontario waters and limited commercial fishing reopened in Ontario (Hatch et al. 1987). Recovery

of the fishery during the late 1970s was attributed to its previous closure, strong year classes in the early 1970s (Nepszy 1977), and improvements associated with the passage of the Great Lakes Water Quality Agreement in 1972 (Roseman et al. 2002). Walleye numbers again declined in the mid through late 1990s, and annual recruitment continues to be highly variable (Vandergoot et al. 2010). The sports fishery harvest was estimated to be ~1.2 million in 2010, while the commercial fishery harvest was ~960 000. Both estimates were below the long-term averages (1975-2010), by 53% and 46% respectively (Lake Erie Committee - Walleye Task Group 2011). As established by the Lake Erie Committee, the total allowable catch decreased from 9 million walleye in 1995 to 2.9 million in 2011 (Lake Erie Committee - Walleye Task Group 2011). Lake Erie walleye in 2011 largely comprise the moderate 2007 and exceptional 2003 year classes (Lake Erie Committee - Walleye Task Group 2011).

Although stocking information in the Great Lakes is incomplete, no stocking history was found for the sites tested in this study. The Great Lakes Fishery Commission's Fish Stocking Database (http://www.glfc.org/fishstocking/) indicates that walleye were stocked in eastern Lake Erie alone by the Pennsylvania Fish and Boat Commission in Conneaut Creek from 2003-2005 (N=11 650) and at Presque Isle Bay in 2001 and 2005 (N=1 800 000). Stepien et al. (2004, with coauthor D. Einhouse from the New York Department of Environmental Conservation) further reported that 2.2 million 1-3 day old fry and 44 000 fingerlings from a hatchery origin from the Maumee River were stocked at Cattaraugus Creek each year from 1995-2000, but found no genetic evidence that the stocking was successful.

1.2 Life history and walleye recruitment

In early spring walleye migrate to spawn, aggregating in tributaries and shallow lake reefs (Ferguson and Derksen 1971; Wolfert and Van Meter 1978). Homing behavior is known to occur in salmonids (Hasler and Scholz 1983; Dittmann et al. 1996), as well as other fish species (Gerking 1955), and has been implicated for walleye (Crowe 1962; Olson et al. 1978). Imprinting occurs during the fishes' early life history and highly developed olfactory systems are used to detect odors to guide homing (Horrall 1981; Gerlach et al. 2001). One hypothesis is that the chemical cues used to identify unique natal streams odors originate from the soils and vegetation (Hasler and Wisby1951), which cause water chemistry to differ among sites (Rondeau et al. 2005). Despite intra-annual variation, water chemistry concentrations of barium (Ba), magnesium (Mg), manganese (Mn) and strontium (Sr) in a given year provide distinct chemical signatures among Lake Erie tributaries (Pangle et al. 2010). The possibility that differences in water chemistry may guide walleye to locate natal spawning sites in Lake Erie merits further investigation.

The female walleye releases thousands of eggs (Regier et al. 1969) at night that settle in crevices and are fertilized by up to a few males (Kerr et al. 1997). No parental care is provided, and after spawning the adults move to adjacent bays and littoral areas and then onto summer feeding grounds (Kerr et al. 1997).

Currents, wave action, and prevailing winds oxygenate the fertilized eggs (Kerr et al. 1997), but these same processes cause some eggs to dislodge and perish (Johnson 1961; Serns 1982; Roseman 2001). The eggs hatch after incubating for approximately three weeks (Kerr et al. 1997), with shorter hatching periods associated with warmer

temperatures and better survival due to less predation and reduced exposure to potentially damaging waves (Johnson 1961; Kerr et al. 1997). The larval yolk sac is absorbed after about five days (Zhao et al. 2009) and the 6.0-8.6 mm larvae then drift to upper limnetic zones (Kerr et al. 1997), where they are dependent on favorable water temperatures and currents to transport to them to productive nursery grounds (Zhao et al. 2009). Young-of-year (YOY) walleye school in nursery areas, where they rely on abundant supplies of zooplankton for food, and are segregated from juvenile and adult walleye (Kerr et al. 1997). As the YOY grow, they become photosensitive and the juveniles then move to deeper water (1-3 m) in late summer, preferring darker habitats with plant cover, where they feed on mayflies, amphipods, and fishes (Scott and Crossman 1973; Kerr et al. 1997). Males generally mature by age 2 and females by age 3 (Hatch et al. 1987).

1.3 Lake Erie basins and walleye spawning sites

Lake Erie is composed of three basins - western, central, and eastern – with the western basin being the shallowest and youngest, consisting of a riverine system until ~4 thousand years ago (kya; Fig. A-1; Hartman 1973; Bolsenga and Herdendorf 1993). Walleye are believed to have colonized the western basin from the Mississippian refugium via the Wabash/Maumee River system (Bailey and Smith 1981). The western basin houses the largest walleye spawning aggregations in Lake Erie (Einhouse and MacDougall 2010), including spawning runs in the Maumee and Sandusky Rivers ~25 km upstream from the rivers' mouths (Mion et al. 1998). The Ballville Dam, constructed in 1911, on the Sandusky River restricts upstream accessibility (Mion et al. 1998). Although the Maumee River has a mean discharge rate about four times greater than the Sandusky River, both have relatively low current velocities and gradients (Mion et al.

1998). They empty into large bays 2-3 m in depth that house nursery grounds (Mion et al. 1998). Spawning in the western basin begins shortly after the ice recedes and peaks the third week of April (Roseman et al. 1996).

The Maumee and Sandusky River spawning sites share similar geological features, which may impart similar chemical signals to guide walleye to spawning sites. Their uppermost bedrock comprises older sedimentary rocks (Casey et al. 1997) of limestone, dolomite, sandstone, and shales in the Maumee River (Casey et al. 1997). The Sandusky River contains the same carbonate rocks, but lacks sandstone bedrock (Casey et al. 1997). The Sandusky River had the highest Sr concentration of the Lake Erie tributaries tested by Pangle et al. (2010), followed by the Maumee River.

Glaciers hollowed the softer shale bedrock in the central and eastern portions of Lake Erie, forming deeper basins (Campbell 1995) and exposing younger sedimentary rocks. The deeper central basin (Hartman 1973) contains limited spawning substrate (Nepszy et al. 1991) and its walleye fishery largely is supported by western basin spawning groups (Einhouse and MacDougall 2010).

The eastern basin is the deepest (Ryan et al. 2003) and oldest basin, dating to ~10kya (Fig. A-1; Bolsenga and Herdendorf 1993), whose walleye spawning aggregations are considered distinct from those in the western basin (Wolfert and Van Meter 1978; Ryan et al. 2003). The smaller eastern basin walleye population (Einhouse and McDougall 2010) is believed to trace its origins to colonization from both the Mississippian and the Atlantic glacial refugia (Stepien and Faber 1998). The inceptisol soils in southeastern Lake Erie are poorly developed, with reduced weathering and lower clay content, which may impart a different chemical signal to fish. Most of its walleye

spawning habitat centers on a reef complex around Van Buren Bay and in several tributaries, including the Grand River, Cattaraugus Creek, and Smoke's Creek, with walleye from the tributaries each being quite genetically divergent (Strange and Stepien 2007; Stepien et al. 2010). Spring warming in the deeper eastern basin trails warming in the western and central basins by several weeks (Hartman 1973), and its walleye runs occur later.

Chapter 2

Materials and methods

2.1 Sample collection

Walleye fin clip samples (1-2 cm^2 of pectoral or caudal fins) were collected for this study by the Ohio Division of Wildlife (ODW) of the Ohio Department of Natural Resources and by the New York Department of Environmental Conservation (NYDEC) during spring spawning runs at three sites: the Maumee River (~41.5611°N, -83.6438°W), the Sandusky River (~41.3498°N, -83.1121°W), and Van Buren Bay (~42.4617°N, -79.3950°W; Fig. A-1). Some agency collectors took sex, length, and otolith age data, which then were used for the present study. After clipping fins, most individuals were released. Fin clips were immersed in 95% ethanol or frozen and subsequently transferred to ethanol. All fin clips were archived and stored at room temperature in the University of Toledo's Great Lakes Genetics Laboratory at the University of Toledo's Lake Erie Research Center.

Samples tested in this study include Maumee River - 1995 (*N*=19 collected on April 8, and *N*=34 April 18), 1998 (*N*=28 March 30), 2003 (*N*=76 collection date unknown), 2007 (*N*=43 March 28), and 2008 (*N*=50 April 7); Sandusky River - 1995

(*N*=41 April 17), 1998 (*N*=50 March 25), 2003 (*N*=30 April 11), 2007 (*N*=32 April 2, and *N*=28 April 10), and 2008 (*N*=36 April 8, and *N*=10 April 22), and Van Buren Bay reefs - 1995 (*N*=33 April 26), 1998 (*N*=51 April 7-23, with most collected April 20-21), 2003 (*N*=57 collection date unknown and *N*=30 collected April 25), 2007 (*N*=28 April 30), and 2008 (*N*=50 collected April 21-28). The 2003 samples included were those from Strange and Stepien (2007) and Stepien et al. (2009, 2010), with ten additional individuals added in this study to both the Sandusky River and Van Buren Bay. Fish were assessed by the agencies as in spawning condition.

Sex data and sufficient numbers of female samples were available from the Maumee River run in 1998 (*N*=11 female, *N*=11 male), 2007 (*N*=19 female, *N*=23 male), and 2008 (*N*=25 female, *N*=25 male), the Sandusky River in 2003 (*N*=15 female, *N*=15 male), 2007 (*N*=29 female, *N*=31 male), and 2008 (*N*=23 female, *N*=23 male), and the Van Buren Bay run in 1998 (*N*=16 female, *N*=34 male); these were used to test for possible genetic differences between the sexes.

Age data were obtained from the ODW (Travis Hartman, 305 E. Shoreline Dr., Sandusky, OH 44870, USA, personal communication) to test for possible genetic differences among cohorts using annual rings on otoliths or from site-specific age-length keys. We restricted analysis to those age cohorts that contained five or more individuals per spawning run sample. Those pairwise analyses included Maumee River run samples collected during the 2007 run that were calculated to have been born in 1999 (*N*=7), 2001 (*N*=6), and 2003 (*N*=18) and those from the 2008 run that were estimated to have been born in 1999 (*N*=6) and 2003 (*N*=10). Also tested were Sandusky River run samples collected during the 2007 run that were estimated to have been born in 2001 (*N*=17),

2003 (*N*=19), and 2004 (*N*=5) and those from the 2008 run that were calculated to have been born in 2003 (*N*=9), 2004 (*N*=8), 2005 (*N*=5), and 2006 (*N*=5).

2.2 DNA extraction and amplification

Genomic DNA was extracted from the fin clips using a DNeasy Qiaquick kit (Qiagen, Inc., Valencia, California). The polymerase chain reaction (PCR) was used to amplify allelic length variants from nine nuclear microsatellite loci previously used in our laboratory by Strange and Stepien (2007) and Stepien et al. (2009, 2010): *Svi*4, *Svi*17, *Svi*18, and *Svi*33 from Borer et al. (1999); *Svi*L6 and *Svi*L7 from Wirth et al. (1999); and *Svi*2, *Svi*6, and *Svi*7 from Eldridge et al. (2002). A 5' fluorescent label was incorporated in each forward primer to allow fragment length visualization on an ABI (Applied Biosystems Inc., Fullerton, California) 3130XL Genetic Analyzer. Multiple dye labels facilitated multi-plexing (combining primers in a single PCR reaction) and pool-plexing (combining results from several PCR runs in a single gel well for simultaneous analysis of multiple loci) to reduce costs.

The PCR reaction mixture contained 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 50 μM of each deoxynucleotide, 0.5 μM of both forward and reverse primers, 5-30 ng DNA template, and 0.6-1.2 units of *Taq* polymerase per 10 μL of reaction volume. All reaction profiles included a 2 min initial denaturation at 94ºC, followed by primer-specific protocols: (a) 40 cycles of denaturation at 95ºC for 1 min, 1 min annealing at 54ºC, and 40 s extension at 72°C for primers *Svi*17, *Svi*L6, and *Svi*L7; (b) 36 cycles of denaturation at 94º C for 30 s, 1 min annealing at 60ºC, and 30 s extension at 72ºC for *Svi*2, *Svi*4, *Svi*6, *Svi*7, and *Svi*33; or (c) 35 cycles of denaturation at 94ºC for 30 s, 1 min

annealing at 63ºC, and 30 s extension at 72°C for *Svi*18. Lastly, all reactions were capped with a 1 min extension at 72°C.

Amplification products were diluted 1:50 with dH_2O, of which 1 µL was added to 13 µL of a solution containing formamide and ABI Gene Scan 500 size standard, and then were loaded onto a 96-well plate. Samples were denatured for 2 min at 95°C prior to loading on an ABI 3130XL Genetic Analyzer in the laboratory. Allelic variants were identified by size using ABI GeneMapper 3.7 software, and output profiles were verified.

2.3 Data analyses

Samples were tested for conformance to Hardy-Weinberg equilibrium (HWE) expectations at each locus, with significance estimated from the Markov chain Monte Carlo (MCMC) method using 10 000 dememorizations, 20 batches and 5000 iterations following Guo and Thompson (1992) in GENEPOP v4.0 (Rousset 2008; http://kimura.univ-montp2.fr/~rousset/Genepop.htm). Loci were tested for linkage disequilibrium (LD) in GENEPOP using 10 000 dememorizations, 20 batches, and 5000 iterations. Because simultaneous tests using multiple loci and multiple populations may increase the probability of falsely rejecting the null hypothesis (type I error), levels of significance for the HWE and LD tests were adjusted using Bonferroni corrections (Sokal and Rohlf 1995). Deviations were evaluated for heterozygote deficiency or excess, and for the possible occurrence of null alleles with MICRO-CHECKER v2.2.3 (van Osterhout et al. 2004, 2006; http://www.microchecker.hull.ac.uk).

Descriptive *F*-statistics of variation included the inbreeding coefficient (F_{IS}), deviation across all samples (F_{IT}), and genetic divergences (F_{ST}) per locus and over all loci using FSTAT v2.9.3.2 (Goudet 2002; http://www2.unil.ch/popgen/softwares/

fstat.htm). Genetic variation parameters per spawning group sample also included observed heterozygosity (H_O) and expected heterozygosity (H_E) using GENEPOP. The total number of alleles (N_A) was determined within ARLEQUIN v3.11 (Excoffier et al. 2005; Excoffier 2007; http://cmpg.unibe.ch/software/arlequin3). Allelic richness (R_A) per spawning group was assessed using FSTAT, and the number of private (unique) alleles was calculated using CONVERT v1.31 (Glaubitz 2004; http://www.agriculture.purdue.edu/fnr/html/faculty/Rhodes/Students%20and%20Staff/glaubitz/software.htm). The percent of private alleles was determined by dividing the number of private alleles (those unique to that spawning group alone) by the corresponding total number of alleles at that spawning group. Parameters were evaluated for each spawning location separately and among spawning sites and years.

Parametric tests using unbiased θ estimates of F-statistics (Weir and Cockerham 1984) were employed to quantify genetic heterogeneity with pairwise tests for population sites, sampling years, the sexes, among age cohorts, and sampling dates within sampling years (1995 in the Maumee River and 2008 in the Sandusky River) using FSTAT. Associated levels of significance were evaluated using ARLEQUIN with 100 000 replicates. Divergence among sample sites was tested by, (a) combining all years to test for overall patterns with a larger sample size and (b) separately to test for differences among the three sites per spawning run year. For tests of individual sites, the means of 10 replicates were taken because some pairwise tests indicated borderline significance. Values of θ_{ST} that differed significantly from zero after sequential Bonferroni correction (Rice 1989) were interpreted as evidence for rejecting the null hypothesis of no difference among sites, years, sex, age cohorts, and/or sampling dates.

Because microsatellite loci often have many alleles and their genotypes may display lower than expected frequencies (Allendorf and Luikart 2007), additional comparative pairwise tests were conducted following the method of Goudet et al. (1996). That method employs an exact nonparametric procedure. Nonparametric tests have less statistical power (Goudet et al. 1996), but are less affected by sample size, and not dependent on a normal distribution (Raymond and Rousset 1995; Rousset 2008). Probabilities were estimated using a Markov Chain Monte Carlo (MCMC) approach in GENEPOP (Rousset 2008) using 10 000 dememorizations, 100 batches and 5000 iterations per batch. Again, sites were tested by combing spawning run years for each of the three sites, by separately testing for differences among sampling sites and runs, and testing for divergence between the sexes and among age cohorts and sampling dates. Results from these pairwise tests were adjusted using sequential Bonferroni corrections (Rice 1989) to minimize type I errors.

Whereas *F*-statistics and contingency tests use the sample location as the unit of comparison, the Bayesian model-based method in GENECLASS2 v2 (Piry et al. 2004; http://www.ensam.inra.fr/URLB/GeneClass2/Installation.htm) uses the individual as the unit, assigning it to the most likely population group(s) regardless of geographic origin. GENECLASS2 used 10 000 simulated individuals and a 0.01 rejection level (Paetkau et al. 2004) to assign each individual fish to the most likely sampling location, i.e., among the three sites, and then (in a separate analysis run) to the most probable sampling site and year.

Hierarchical partitioning of genetic variation (% variance) among groups of populations, population samples within groups, and variation within population samples

then were evaluated using AMOVA (Analysis of Molecular Variance; Excoffier et al. 1992) in ARLEQUIN. Separate hierarchical partitioning runs tested possible grouping scenarios: (a) among the three spawning site locations and their sampling years, (b) among sampling years and the three spawning site locations, (c) between the sexes and the spawning site locations (for available data) within them, (d) between the cohorts born in 2001 and 2003 (where age data were available from both the Maumee and Sandusky River runs) and the two spawning sites within them, and (e) between the Maumee and Sandusky River run spawning sites and their cohorts born in 2001 and 2003. Additionally, 3-Dimensional Factorial Correspondence (3DFCA) analysis in GENETIX v4.05 (Belkhir et al. 2004; http://www.genetix.univ-montp2.fr/genetix/intro.htm) was used to visualize the overall genetic relationships among spawning sites and years.

Chapter 3

Results

3.1 Genetic composition

Following Bonferroni correction, the population samples at each of the nine loci did not deviate significantly from Hardy-Weinberg equilibrium (HWE) expectations, were discerned to be unlinked, and showed no overall evidence of null alleles. The total number of alleles was 132, averaging ~15 per locus (ranging from seven for *Svi*18 to 25 for *Svi*L7; Table A.1). Loci that contributed the most to divergence among samples, as measured by $F_{ST,}$ were *Svi*2 and *Svi*L6 (Table A.1).

Walleye spawning groups overall had approximately similar numbers of alleles (N_A): 108 for the Maumee River (mean=82, range=76-88 per annual run), 115 for the Sandusky River (mean=86, range=76-96), and 112 for the Van Buren Bay reefs group (mean=84, range=72-89; Table A.2). Overall observed heterozygosity level (H_O) was 0.710 for walleye spawning in the Maumee River (ranging from 0.700-0.724 in individual years), 0.734 for the Sandusky River (0.707–0.762), and 0.751 for Van Buren Bay reefs (0.710–0.801), and thus increased slightly from west to east (Table A.2). Overall allelic richness (R_A) averaged across the nine loci was 11.858 (ranging from

7.794-8.418 in individual years) for walleye spawning in the Maumee River, 12.747 (8.128-9.022) for the Sandusky River, and 12.248 (7.834–9.172) in Van Buren Bay (Table A.2). All spawning groups housed some private alleles (N_{PA}), i.e., alleles that were unique to a site, totaling three in Maumee River (ranging from 0-2 detected per annual spawning run), eight for the Sandusky River (0-3), and six in Van Buren Bay (0-4). Walleye spawning in the Sandusky River had the highest overall proportion of private alleles (P_A=0.070), followed by Van Buren Bay (0.054), with the least in the Maumee River (0.028). Van Buren Bay in 1995 had the highest overall number (4) and proportion (0.045) of private alleles for a site and individual sampling year (Table A.2).

3.2 Temporal genetic patterns within spawning locations

Tests for pairwise genetic differences among annual walleye spawning runs in the Maumee River averaged θ_{ST}=0.002 (ranging from 0.001-0.004, p=0.106-0.999), showing no significant differences. Contingency test results were congruent. The Sandusky River spawning group had a mean pairwise divergence of θ_{ST}=0.004 among the five sampling years (ranging from 0.001-0.010, p=0.004–0.827); no differences were found, since two marginal comparisons lost significance following sequential Bonferroni correction (2007 vs.1995 and 2003). One comparison (1998 vs. 2007) remained significant following sequential Bonferroni correction in the contingency test alone. Pairwise temporal comparisons for the Van Buren Bay spawning location years averaged θ_{ST}=0.005 (ranging from 0.001-0.013, p=0.001-0.864). Five θ_{ST} comparisons differed at p=0.05 (2003 vs. 2007 and 1995 vs. 1998, 2003, 2007, and 2008), but none were significant following sequential Bonferroni correction. Those comparisons remained significant following sequential Bonferroni corrections in the contingency tests alone (Table A.3).

AMOVA results, like the θ_{ST} results, showed no overall significant divergences among sampling years at the three sites (%variation=0.01, p=0.780; Table A.4).

There also were no significant differences in the genetic compositions between males and females at spawning sites tested, shown by the pairwise tests (mean θ_{ST}=0.002, range<0.001-0.008, p=0.059-0.913; mean X^2=18.0, range=10.8-32.2, p=0.021- 0.901) and AMOVA (Table A.4). Similarly, pairwise tests showed that the genetic compositions of age cohorts did not differ in the Maumee River runs in 2007 (mean θ_{ST}<0.005, range<0.001-0.012, p=0.249-0.885; mean X^2=18.5, range=12.8-23.1, p=0.186- 0.805) and 2008 (θ_{ST}<0.001, p=0.856; X^2=15.2, p=0.649), or in the Sandusky River runs in 2007 (mean θ_{ST}<0.005, range<0.001-0.011, p=0.219-0.488; mean X^2=17.9, range=15.4-20.1, p=0.327-0.635), or 2008 (mean θ_{ST}<0.007, range<0.001-0.025, p=0.041-0.917; mean X^2=14.7, range=10.5-23.1, p=0.188- 0.914). A single θ_{ST} pairwise comparison appeared significant at p<0.05, which lost significance after Bonferroni correction (i.e., the 2003 vs. 2004 cohorts in the Sandusky River 2008 spawning run, θ_{ST}=0.025, p=0.041). AMOVA comparisons were congruent, showing no differences among age cohorts (Table A.4). Pairwise tests for walleye spawning in the Maumee River in 1995 that were collected on April 8, and those that were collected on April 18, did not differ significantly (θ_{ST}=0.001, p=0.345). Likewise, pairwise tests for walleye spawning in the Sandusky River in 2008 that were collected on April 2, and April 10, did not differ significantly (θ_{ST}<0.001, p=0.615). Contingency tests were congruent. Thus, overall no significant differences occurred between the sexes or sampling dates or among the age cohorts.

3.3 Temporal genetic relationships among spawning locations

Figure A-2 shows the relationships among walleye spawning groups in the Maumee and Sandusky Rivers and Van Buren Bay, with all years combined (a) and separately for each sampling site and year (b). Overall results showed that the genetic composition of walleye spawning at the three sites significantly differ from each other (θ_{ST}=0.001-0.006, p=0.001-0.047; Table A.3). Contingency tests were congruent. Individual spawning year comparisons show some variation. Results from individual years did not differentiate between the Maumee and Sandusky samples (average θ_{ST}=0.002, p=0.074-0.370). Contingency test results were congruent except for 2008 between the Maumee and Sandusky River samples, which showed a significantly difference (X^2=31.3, p=0.027) following sequential Bonferroni correction.

In 1995 and 1998, the two western basin river spawning groups each showed significantly divergence from the Van Buren Bay group (average θ_{ST}=0.012, p<0.001-0.005), with contingency test results congruent. The Maumee River and Van Buren Bay spawners in 2003 and 2007 differed at p=0.05, which lost significance following sequential Bonferroni correction. The comparisons remained significant post-correction with the contingency tests. The 2003 Sandusky River and Van Buren Bay spawners appeared similar between pairwise θ_{ST} tests, but differed before and after sequential Bonferroni correction in the contingency tests. In 2007, all test results distinguished the Sandusky River and Van Buren Bay spawning groups. In 2008, F_{ST} analogs results showed that spawning groups in the Maumee and Sandusky Rivers appeared more genetically similar to the Van Buren Bay spawning group, with contingency tests

indicating significant divergence following sequential Bonferroni correction (Fig. A-2; Table A.3).

AMOVA tests discerned significant partitioning of genetic variation among the three spawning locations, (Table A.4), consistent with results from the pairwise tests (Table A.3). Partitioning of variation among sampling years was not significant (4b), but variation was significant within the spawning sites (4a). Overall, there was much greater variability among the three spawning sites (0.38-0.56%) than among sampling years (0.01-0.22%; Table A.4).

The 3DFCA (Fig. A-3) explained ~50% of the overall genetic variation among the sampling sites and years. Results showed that Van Buren Bay spawning walleye were more divergent, with its 1995 sample being widely separate – as was found in the pairwise tests (Table A.3). Samples spawning in the Maumee River and the Sandusky River appeared genetically similar, as shown in the results from the pairwise comparisons among individual spawning years, but differed significantly in the overall combined year samples (Fig. A-2).

GENECLASS assignment tests among the three spawning sites showed that walleye spawning in the Sandusky River and Van Buren Bay runs assigned back to their respective sites more often than to any other site; however, walleye spawning in the Maumee River assigned most often to Van Buren Bay (Table A.5). Considering possible assignment to three or 15 groups respectively (comparisons among the three spawning sites, and among all years per sites), 22% and 33% of walleye spawning in the Maumee River runs genetically assigned back to the Maumee River, most of those spawning in the

Sandusky River (56% and 65%) self-assigned, and almost all those spawning in Van Buren Bay reefs (91% and 80%) self-assigned (Table A.5).

Chapter 4

Discussion

The total number of alleles in the present study of 726 individuals spawning in the Maumee and Sandusky Rivers and Van Buren Bay reefs was comparable to the number reported by Strange and Stepien (2007) from 448 spawning individuals spawning at 10 Lake Erie spawning sites and a single Lake St. Clair site. The number of alleles found here also was similar to that reported by Stepien et al. (2009) for 801 walleye individuals at 20 spawning sites throughout the Great Lakes and their tributaries and 921 individuals in six watersheds in North America. Heterozygosity levels ranging from 0.710-0.751 fit values reported by Strange and Stepien (2007) of mean=0.707 (range 0.660-0.780) averaged across 11 Lake Erie sites. Thus, this study found appreciable genetic diversity in walleye that spawn in the Maumee and Sandusky Rivers and Van Buren Bay reefs. Heterozygosity levels also were consistent across sampling years, suggesting stable genetic diversity of walleye spawning at each of the three locations.

Although this study found a similar proportion of private alleles in walleye that spawned in the Maumee River and Van Buren Bay reefs as described by Strange and Stepien (2007), a greater proportion of private alleles was detected in the Sandusky River

here. The present study found a single private allele in the Sandusky River 2003 spawning group, and identified three additional private alleles in both 1998 and 2008 samples. Sample sizes in Strange and Stepien (2007) and Stepien et al. (2009) were *N*=76 for the Maumee River and *N*=77 for the Van Buren Bay reefs, while the Sandusky River sample size was only *N*=20; this sample size was increased to 30 here. Hence, a single year snap shot and the lower sample size in the Sandusky River did not capture these additional private alleles.

Walleye spawning in the Maumee and Sandusky Rivers showed little intra-site genetic divergence from year to year, while those spawning in Van Buren Bay reefs in 1995 differed from the other years. The genetic composition of males versus females did not differ at given spawning locations. Genetic similarity of males and females was also found for mtDNA analyses of the 1993 and 1995 walleye runs across Lake Erie (Stepien and Faber 1998); likewise, no differences were found between the sexes at a variety of walleye spawning sites using microsatellite data by Strange and Stepien (2007) and Stepien et al. (2009, 2010). There also were no differences among age cohorts. It is possible that limited availability of age data might have constrained ability to detect genetic differences among some age cohorts. Thus, results suggest male and female walleye and age cohorts exhibit similar degrees of natal site fidelity that appear consistent from year to year and likely from generation to generation.

Walleye spawning at a given site that were sampled on different days of the run also appeared genetically similar, but comparisons were limited to two sites and further testing is necessary. Relationships within spawning sites thus appear stable; this pattern remains largely consistent in genetic composition for each of the three spawning sites

from year to year and consistent between sexes, among age cohorts, and between sampling dates.

The genetic relationships among walleye spawning in the Maumee and Sandusky Rivers and Van Buren Bay reefs appeared to vary in some years, although observed variances were likely due to limited samples sizes and relatively low degree of genetic divergences. Waples (1998) found that when samples sizes were increased from 25 to 50, 100, and 200 that the signal to noise ratio in F_{ST} values decreased to 50%, 25% and 12.5%, respectively. Ruzzante (1998) compared the effects of two versus six loci and sample size on F_{ST}, finding greater variance with small sample sizes ($N<50$) and relatively little effect at sample sizes greater than 100. Ruzzante (1998) suggested that sample sizes should be between 50-100 using microsatellite markers; likewise Kalinowski (2005) indicated that larger sample sizes are needed when the amount of divergence is small (as found in the present study). Using simulated data and four loci, Kalinowski (2005) concluded that 20 individuals per sample were sufficient when θ_{ST} values were greater than 0.05 and 100 individuals were needed for values less than 0.01 (values here are in that lower range). Ryman et al. (2006) found that total sample sizes (subpopulations x number of individuals) of 250 had greater power than total sample sizes of 100 in comparisons of F_{ST} values of 0.001 using 10 loci.

These studies suggest that the noise to signal ratio is less and that there is greater power in comparisons in this study among the three sites when all years are combined. Sample sizes >100 are needed to test divergence in further comparisons. As demonstrated by Ryman et al. (2006), the present study found that comparisons among sites using individual years had much lower power due to low sample size and small F_{ST} values,

versus analyses that combined years for greater sample sizes. Consistent with Kalinowski's (2005), all comparisons in the individual years with F_{ST} analogs ~0.01 showed significant genetic divergence at lower sample sizes and those with F_{ST} <~0.01 in individual years were not significant. After years were combined for the spawning sites and sample sizes reached >100, the sampling sites showed significant divergence. The inability to discern divergence with F_{ST} analogs less than 0.01 with fewer than 100 individuals may also explain differences between significant θ_{ST} and contingency values in individual years. In each instance, θ_{ST} values were between 0.005 and 0.013, with samples sizes less than 100. The ability to distinguish genetic signal also is enhanced by using multiple loci (Waples 1998). Ruzzante (1998) found that sample variances decreased when the number of loci was increased from two to six. Here a moderate number of loci were used. It appears likely that larger samples sizes are needed to consistently evaluate genetic differences among these three sites.

The Van Buren Bay reef spawning group in the eastern basin appears most divergent, as suggested by F_{ST} values and higher self-assignment in the GENECASS analysis. This is consistent with other walleye studies in our lab that found greater genetic divergence in most eastern Lake Erie basin walleye spawning sites (Strange and Stepien 2007; Stepien et al. 2009), as well as for smallmouth bass *Micropterus dolomieu* (Stepien et al. 2007) and greenside darter *Etheostoma blennioides* (Haponski and Stepien 2008). Although the Van Buren Bay reefs site is geographically distant from the Maumee and Sandusky River spawning sites, prior spatial analyses (including other Lake Erie spawning groups) showed that these relationships did not conform to a genetic isolation with geographic distance pattern and found that some more closely spaced spawning sites

were more divergent than were others (Strange and Stepien 2007; Stepien et al. 2009, 2010). Thus, observed genetic patterns in Lake Erie walleye appear more complex than explained by genetic isolation with geographic distance.

The closer genetic relationships between walleye spawning groups in the Maumee and Sandusky Rivers also may be due to their physical, biological, and chemical similarities. For example, spawning activity in Van Buren Bay reefs lags behind spawning activity in the shallower, warmer western basin and temporal isolation among spawners may restrict gene flow. Similar vegetation, soil and bedrock types in the Maumee and Sandusky Rivers may lead to common homing to these environmental cues using olfactory senses.

The closer genetic relationship between spawning groups in the Maumee and Sandusky Rivers appears consistent from year to year and may suggest that a single genetic group spawns across these two riverine sites. However, very few walleye spawning in the Sandusky River genetically assigned to the Maumee River. Also, when overall sample sizes were increased (by combining all years per site) significant genetic differences were found. Genetic divergence between walleye spawning groups in the Maumee and Sandusky Rivers also were found in a prior study using mitochondrial DNA control region restriction fragment length polymorphisms (RFLPs) from spawning runs in 1992 and 1993 (Merker and Woodruff 1996). Recolonization in the western basin after the 1970s population crash also could make it difficult to distinguish these groups today, as variation at neutral loci can take thousands of generations to recover following a reduction of population size (Milligan et al. 1994).

Interestingly, walleye spawning in the Maumee River consistently had the lowest overall genetic self-assignments, with most individuals from 2003 assigning to the Van Buren Bay reefs spawning group. Strange and Stepien (2007) detected higher gene flow between walleye spawning in the Maumee River and Van Buren Bay reefs than between any of the other sites (*N*=11) tested. It is unclear whether the observed pattern of connectivity between these sites is contemporary in nature (i.e., a result of immigration of walleye from the Van Buren Bay spawning group into the Maumee River), or reflects historic immigration (i.e., a relict of colonizations from glacial refugia). These hypotheses remain to be tested.

To summarize, (1) population genetic compositions of walleye spawning groups are largely consistent from year to year, with some evidence for genetic divergence among some years in Van Buren Bay reefs. (2) The degree of genetic connectivity and divergence relationships among walleye spawning groups are consistent in that the Maumee and Sandusky Rivers appear more similar to each other than to Van Buren Bay from year to year and inconsistent in that the genetic connectivity between the riverine sites and the Van Buren Bay reef site differs in some years and not in others, which likely is a function of sample size. The three spawning locations overall appear divergent. (3) The genetic composition of males versus females does not differ within a given spawning run. (4) The genetic composition of age cohorts does not appear to differ within a given spawning run, but additional testing and larger samples sizes are suggested. (5) The genetic composition between sampling dates in a given year does not appear to differ within spawning runs, but also should be further tested.

Although genetic divergences in this study were moderate, they often are underestimated in large open systems with high levels of connectivity (Reiss et al. 2009; Conover et al. 2006). Also, only a few migrants per generation are needed to eliminate evidence of genetic differences between spawning groups (Moran 2002). Thus levels of divergences among spawning groups in Lake Erie are likely conservative, especially between the geographically close Maumee and Sandusky River spawning sites. Evidence of divergence presented here corresponds with differences in habitat, homing behavior, and olfactory chemistry. Results add to mounting evidence suggesting that walleye spawning groups in Lake Erie are demographically independent and as such should be managed independently to avoid local reductions of individual stocks and to maintain sustainable lake wide harvests.

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Appendix A

Tables and Figures

Table A.1: Summary of genetic variation per microsatellite locus across the three spawning groups of walleye (N=726 individuals, 5 sampling years, 3 spawning sites), showing number of alleles (N_A), allelic size range (base pairs, bp), inbreeding coefficient (F_{IS}, average genetic divergence within a spawning group), genetic deviation across all combined samples (F_{IT}), and mean genetic divergence among the three spawning groups (F_{ST}).

Locus	N_A	Size range (bp)	F_{IS}	F_{IT}	F_{ST}
*Svi*2	19	174-226	-0.002	0.010	0.012
*Svi*4	10	104-122	0.005	0.014	0.009
*Svi*6	19	128-172	0.017	0.018	0.001
*Svi*7	11	154-176	-0.015	-0.017	-0.002
*Svi*17	10	100-118	0.016	0.018	0.003
*Svi*18	7	116-128	0.091	0.094	0.003
*Svi*33	14	80-106	-0.022	-0.021	0.002
*Svi*L6	17	106-140	0.074	0.087	0.014

*Svi*L7	25	174-236	0.038	0.039	0.001
Total (Mean)	132	--------	(0.021)	(0.026)	(0.005)

Table A.2: Genetic variation of walleye spawning groups (total from all years sampled, mean of the five years sampled, and per individual sampling year): metrics include number of individuals (N), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), total number of alleles (N_A), mean allelic richness across all loci (R_A), number of private alleles (N_{PA}), and proportion of private alleles to the total number of alleles at each location and year (P_{PA}).

Site and year	N	H_O	H_E	F_{IS}	N_A	R_A	N_{PA}	P_{PA}
Maumee R., total	250	0.710	0.734	0.033	108	11.858	3	0.028
Mean of 5 years	50	0.712	0.735	0.031	82	8.091	<1	0.007
1. 1995	53	0.724	0.743	0.026	88	8.418	0	0.000
2. 1998	28	0.721	0.731	0.013	76	8.225	2	0.026
3. 2003	76	0.700	0.731	0.042	87	8.084	1	0.011
4. 2007	43	0.712	0.730	0.024	79	7.934	0	0.000
5. 2008	50	0.701	0.738	0.050	81	7.794	0	0.000
Sandusky R., total	227	0.734	0.742	0.010	115	12.747	8	0.070
Mean of 5 years	45	0.737	0.742	0.006	86	8.419	<2	0.018
6. 1995	41	0.729	0.743	0.019	82	8.195	1	0.012
7. 1998	50	0.754	0.748	-0.008	96	9.022	3	0.031
8. 2003	30	0.762	0.761	-0.002	76	8.128	1	0.013
9. 2007	60	0.707	0.728	0.028	92	8.402	0	0.000
10. 2008	46	0.735	0.731	-0.005	85	8.348	3	0.035
Van Buren Bay	249	0.751	0.769	0.024	112	12.248	6	0.054
Mean of 5 years	50	0.756	0.767	0.015	84	8.324	<2	0.014

11. 1995	33	0.786	0.782	-0.006	89	9.172	4	0.045
12. 1998	51	0.710	0.759	0.064	84	8.221	0	0.000
13. 2003	87	0.761	0.773	0.016	88	8.244	0	0.000
14. 2007	28	0.801	0.769	-0.041	72	7.834	0	0.000
15. 2008	50	0.724	0.754	0.040	85	8.149	2	0.024

Table A.3: Pairwise tests of genetic divergence between walleye spawning samples (a) with all sampling years combined for a given site and (b) per sampling year, showing F_{ST} analog θ_{ST} values below the diagonal and X^2 contingency test results above the diagonal. *Italics*=significant at 0.05 level, underlined=significant following sequential Bonferroni correction for 105 comparisons, all others=not significant, and Inf=infinite X^2 from program output. [Note that the difference between the significance of corrected value between Maumee and Sandusky Rivers 1995 and Maumee River and Van Buren Bay 2003 and 2008 in Fig. A-1 is due to the latter's basis on 3 simultaneous comparisons versus the 105 here, for which Table b likely reflects an over-correction].

(a)

Location (all years combined)	Maumee R.	Sandusky R.	Van Buren B.
1. Maumee R	------	*29.9*	*Inf*
2. Sandusky R.	*0.001*	------	*Inf*
3. Van Buren Bay	*0.005*	*0.006*	------

(b)

Location and year	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Maumee R '95	------	27.7	20.4	28.0	*36.2*	27.4	14.0	21.2	*Inf*	27.6	*Inf*	*54.1*	*65.2*	*47.9*	*39.9*
2. Maumee R '98	0.001	------	*32.7*	26.1	*33.2*	*39.0*	16.1	*38.9*	*36.5*	25.3	*45.1*	*52.3*	*52.8*	*52.7*	*45.4*
3. Maumee R '03	0.001	0.002	------	19.0	*32.3*	17.8	12.1	26.0	*Inf*	22.4	*Inf*	*47.2*	*40.3*	*57.4*	24.3
4. Maumee R '07	0.001	0.002	0.001	------	13.3	11.3	13.6	23.9	23.6	15.9	*Inf*	*33.7*	*47.9*	*36.1*	24.1
5. Maumee R '08	0.002	0.004	0.001	0.001	------	24.9	25.4	*31.8*	*42.3*	*31.3*	*Inf*	*50.3*	*Inf*	*49.7*	*45.9*
6. Sandusky R '95	0.004	*0.009*	0.001	0.001	0.001	------	12.3	24.6	*31.5*	19.4	*Inf*	*43.7*	*57.4*	*39.1*	26.5
7. Sandusky R '98	0.001	0.001	0.001	0.001	0.001	0.001	------	22.3	*Inf*	16.0	*Inf*	*39.2*	*42.8*	*42.8*	20.9
8. Sandusky R '03	0.002	*0.011*	0.002	0.003	0.001	0.002	0.003	------	*34.7*	27.8	*Inf*	*30.9*	*38.6*	*41.8*	*30.9*
9. Sandusky R '07	*0.009*	*0.009*	*0.007*	0.001	0.001	*0.007*	0.004	*0.010*	------	27.0	*Inf*	*58.5*	*Inf*	*45.9*	*Inf*
10. Sandusky R '08	0.004	*0.008*	0.001	0.001	0.001	0.003	0.001	0.002	0.004	------	*Inf*	*41.3*	*67.6*	*49.8*	*32.5*
11. Van Buren B '95	*0.017*	*0.014*	*0.015*	*0.016*	*0.016*	*0.011*	*0.013*	*0.008*	*0.028*	*0.022*	------	*Inf*	*Inf*	*Inf*	*Inf*
12. Van Buren B '98	*0.006*	*0.011*	*0.006*	0.004	*0.005*	*0.006*	*0.008*	0.001	*0.015*	*0.008*	*0.006*	------	*41.1*	*44.8*	*29.6*
13. Van Buren B '03	*0.006*	*0.012*	*0.003*	0.003	*0.006*	*0.004*	0.003	0.002	*0.013*	*0.007*	*0.008*	0.002	------	*Inf*	22.2
14. Van Buren B '07	*0.008*	*0.017*	*0.011*	*0.008*	0.006	*0.012*	*0.011*	0.005	*0.012*	*0.013*	*0.013*	0.005	*0.005*	------	*31.0*
15. Van Buren B '08	0.002	*0.008*	0.002	0.001	0.004	*0.006*	0.001	0.002	*0.011*	0.004	*0.012*	0.001	0.001	0.001	------

Table A.4: Analyses of Molecular Variance (AMOVA) showing the hierarchical distribution of genetic variability among walleye samples grouped by: (a) the three spawning sites (Maumee River, Sandusky River, and Van Buren Bay), and then partitioned by their component years (1995, 1998, 2003, 2007, and 2008), (b) the five sampling years, and then partitioned among the three sites, (c) between the sexes and then partitioned among the sampling sites, (d) between the 2001 and 2003 age cohorts and then partitioned between the Maumee and Sandusky River runs, and (e) between the Maumee and Sandusky River runs and then partitioned between two age cohorts (2001 and 2003). Underlined=significant and all others=not significant. *Maumee River 1998, 2007 and 2008; Sandusky 2003, 2007 and 2008; and Van Buren Bay 1998 were used (Sex for other sampling sites and years either were not available or had too few females).

	Source of Variation	% Variation	Fixation Index	Significance
(a)	1. Among three sampling sites (Maumee R., Sandusky R., Van Buren B.)	0.38	0.004	<u>0.001</u>
	2. Among sampling years (1995, 1998, 2003, 2007, 2008) within the three spawning sites	0.22	0.002	<u>0.001</u>
	3. Within samples	99.40	0.006	<u>0.001</u>
(b)	1. Among five sampling years (1995, 1998, 2003, 2007, 2008)	0.01	0.001	0.780
	2. Among three sampling sites (Maumee R., Sandusky R., Van Buren B.)	0.56	0.006	<u>0.001</u>
	3. Within samples	99.43	0.005	<u>0.001</u>
(c)	1. Between sexes	0.01	0.001	0.972
	2. Among sampling sites* for males,	0.38	0.004	<u>0.001</u>

		females			
	3.	Within samples	99.61	0.003	<u>*0.001*</u>
(d)	1.	Between age cohorts (those born in 2001 and 2003)	0.01	0.001	0.654
	2.	Between sampling sites (Maumee R., Sandusky R.) within age cohorts	0.49	0.005	0.146
	3.	Within samples	99.50	0.004	0.156
(e)	1.	Between two sampling sites (Maumee R. and Sandusky R.)	0.11	0.001	0.452
	2.	Between age cohorts (2001 and 2003) within the two sites	0.33	0.003	0.252
	3.	Within samples	99.56	0.004	0.143

Table A.5: GENECLASS analysis showing the number and percentage (in parentheses) of walleye that assign to a given spawning site, using a simulated population size of 10 000 individuals per site and a rejection level of 0.01. Results from (a) spawning sites, with all sampling years (1995, 1998, 2003, 2007, and 2008) combined and (b) for spawning sites and individual sampling years. **Bold**=number that self-assign and underlined=highest number of individuals assigned to a given group.

(a)

Location (all years combined)	**Maumee R.**	**Sandusky R.**	**Van Buren Bay**
A. Maumee R.	**55**	68	127
	(22%)	(27%)	(51%)
B. Sandusky R.	9	**127**	91
	(4%)	(56%)	(40%)
C. Van Buren B.	5	17	**227**
	(2%)	(7%)	(91%)

(b)

Location	Year	Maumee R.	Sandusky R.	Van Buren B.
Maumee R.	1995	**23**	16	14
		(43%)	(30%)	(26%)
	1998	**16**	5	8
		(55%)	(17%)	(28%)
	2003	**11**	24	41
		(15%)	(32%)	(54%)
	2007	**15**	14	14
		(35%)	(33%)	(33%)
	2008	**18**	16	16
		(36%)	(32%)	(32%)
Sandusky R.	1995	3	**25**	13
		(7%)	(61%)	(32%)
	1998	3	**35**	12
		(6%)	(70%)	(24%)
	2003	2	**20**	8
		(6%)	(67%)	(27%)
	2007	6	**33**	21
		(10%)	(55%)	(35%)
	2008	3	**35**	9

		(7%)	(76%)	(20%)
Van Buren B.	1995	0	0	**33**
		(0%)	(0%)	(100%)
	1998	7	9	**35**
		(14%)	(18%)	(69%)
	2003	7	5	**75**
		(8%)	(6%)	(86%)
	2007	1	4	**23**
		(4%)	(14%)	(82%)
	2008	6	10	**34**
		(12%)	(20%)	(68%)

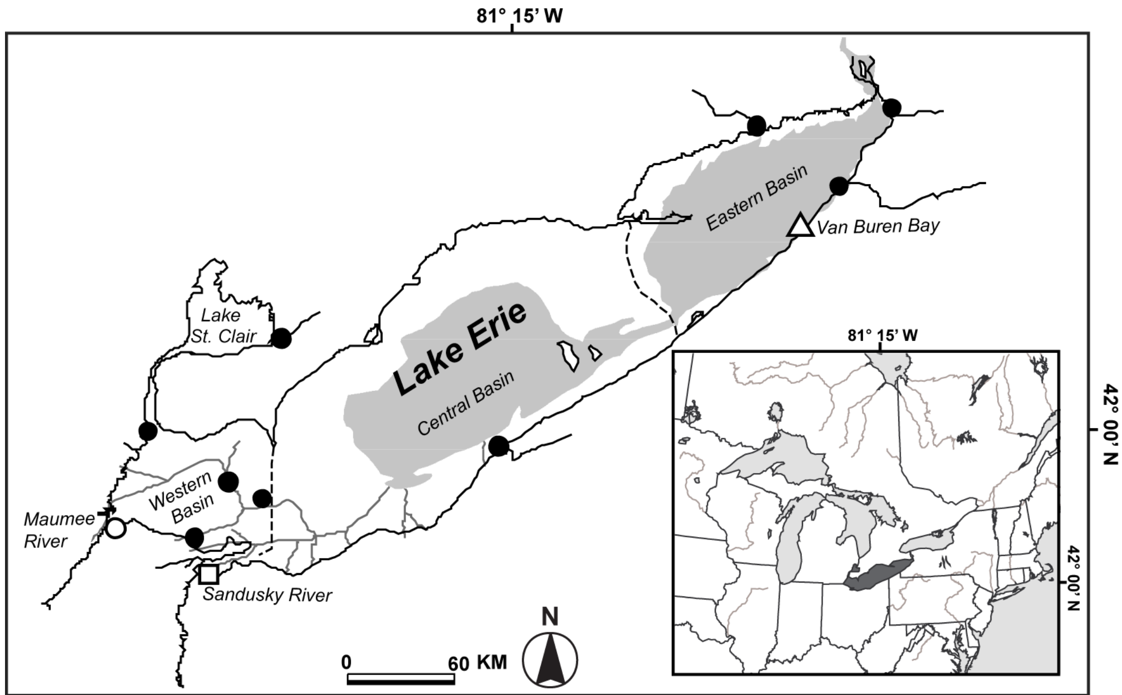

Fig. A-1: Walleye spring spawning sites sampled in Lakes Erie and St. Clair (symbols) by Strange and Stepien (2007) and Stepien et al. (2010), with shaded areas illustrating Lake Erie's shoreline and the riverine system in the western basin ~10 000 years before present (after Bolsenga and Herdendorf 1993 and Strange and Stepien 2007). Dashed lines indicate basin boundaries and open symbols are spawning groups used in present temporal study.

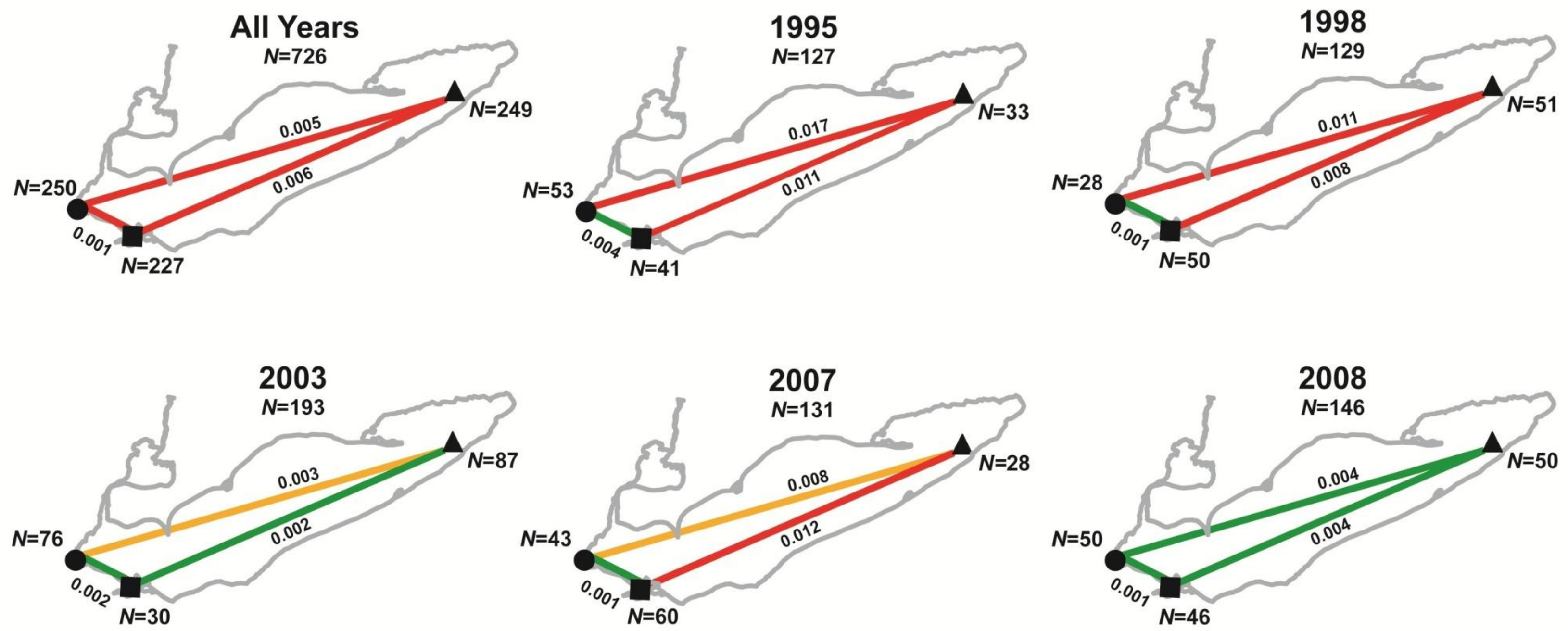

Fig. A-2: Genetic relationships among walleye spawning groups (Maumee R.= ●, Sandusky R.= ■, and Van Buren B.=▲) in Lake Erie for combined sampling years and per year, showing *N*=number of individuals, F_{ST} analog divergences between sites (values along lines), and relative level of significance between pairs of the three sites (not sig.=green, sig. at 0.05=yellow, and sig. after sequential Bonferroni correction=red). Note: F_{ST} comparison values are shown on the line with significance values per sampling year (Bonferroni correction was done per year, i.e., p=0.05/3=p=0.015 in contrast to Table A.3(b), which was corrected for the total number of pairwise comparisons (105).

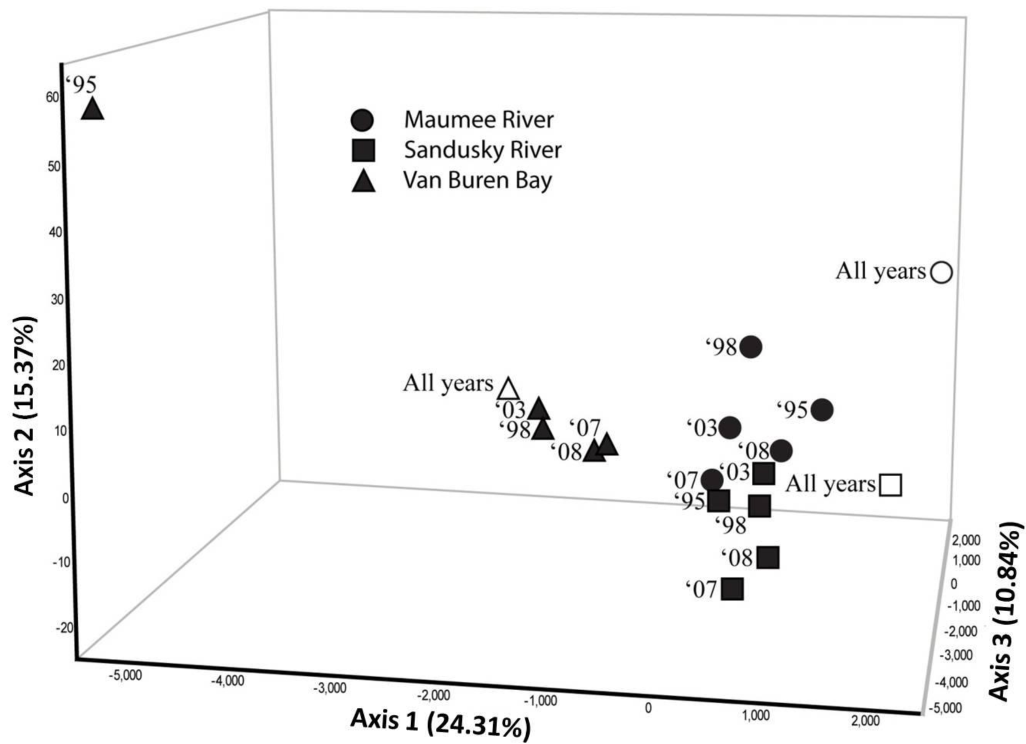

Fig. A-3: Three-dimensional factorial correspondence analysis of relationships among walleye spawning groups in the Maumee and Sandusky Rivers and Van Buren Bay reefs for the years 1995, 1998, 2003, 2007, and 2008. Open symbols designate combined data for five sampling years at that spawning site.